

PROJECT NUMBER: 6908
PROJECT TITLE: Smoke Condensate
PROJECT LEADER: A. H. Warfield
PERIOD COVERED: May, 1990

I. REMOVAL OF TSNA PRECURSORS

- A. **Objective:** To develop methods for the removal of TSNA precursors from filler.
- B. **Results:** It has been hypothesized that calcium incorporated in the tobacco matrix may be associated with precursors of MS TSNA, and that calcium removal may result in reductions of these precursors. To test this hypothesis, DBC Burley (Bu) has been washed with aqueous acetic acid (1*N*) and lanthanum chloride (0.1*N*) solutions in attempts to remove calcium. Analytical data from these experiments are incomplete.
- C. **Plans:** Complete data analysis of the washed fillers and initiate mineral acid washes if the acetic acid and/or lanthanum washes prove to be ineffective at calcium removal.
- D. **References:**

Haut, S. A. Notebook No. 8891, p. 82.

II. NITROSATING AGENT INHIBITORS

- A. **Objective:** To reduce the levels of pyrosynthesized TSNA by interfering with the nitrosating agent(s).
- B. **Results:** In an attempt to investigate the utility of a possible nitrosating agent scavenger, phytymethyl oxalate (PMO) was added to a Bu filler which had been extracted with 5% ethanol in hexane to remove nicotine and preformed TSNA. PMO, obtained from Dr. Rett Southwick, is a known phytadiene release agent. MS TSNA delivered by the sample with added PMO showed no significant reduction in MS TSNA when compared with the control.
- C. **Plans:** Obtain gas phase data from CTD to determine whether NO was reduced in the treated sample.

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D. References:

Haut, S. A. Notebook No. 8891, p. 82.

III. UNEXTRACTED NICOTINE STUDIES

- A. **Objective:** To determine whether there is a correlation between unextracted nicotine (UN) in filler and the delivery of NNK in the corresponding MS smoke.
- B. **Results:** Experiments were carried out in which various enzymes were utilized to convert Nic-Y in water-washed SCFE burley filler to water-soluble forms of UN. The water-soluble Nic-Y would then be analyzed as Nic-X. Therefore, the measure of a successful treatment would consist of a significant *increase* in Nic-X along with a corresponding *decrease* in Nic-Y relative to data from a control experiment in which the enzyme was left out.

An initial study was completed in which cellulase, β -glucosidase, and pronase E were used to digest the filler for 24 hr at 35°C, compared to water-treatment under the same conditions. Cellulase and pronase E gave higher Nic-X levels, but pronase E also gave higher Nic-Y levels. The Nic-Y level for the cellulase sample was not obtained due to experimental difficulties. Pronase E appeared to be the best choice. However, the increases in Nic-X were very small relative to the amount of Nic-Y found in the samples. An additional study was carried out in which proteolytic enzymes were compared to the same treatment minus the enzymes. The enzymes used were Nagarse Type VII, Protease Type XXI, Subtilisin Carlsberg, and Trypsin, as well as Pronase E. Nagarse and Subtilisin Carlsberg gave significant decreases in Nic-Y along with corresponding increases in Nic-X, with Nagarse providing the best results. Although these changes again were small relative to the levels of Nic-Y, the proteolytic enzymes appeared to be the most probable choices thus far for use in a future preparative attempt to isolate a water-soluble modification of Nic-Y.

- C. **Plans:** Additional enzyme digestion experiments will be performed in attempts to optimize the liberation of Nic-Y for isolation.
- D. **Reference:** Lambert, E. A. Notebook No. 8852, pp. 166-169.
- E. **Abbreviations/Definitions:** Nic-X = hot water-soluble alkali-releasable nicotine.
Nic-Y = hot water-insoluble alkali-releasable nicotine.

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IV. ANALYTICAL PROCEDURES

- A. **Objective:** To develop and maintain analytical methodology for TSNA or other compound classes relevant to the nitrosamine content of smoke or filler.
- B. **Results:** Further work has been done on an analytical method for the determination of pseudo-oxy nicotine (PsON). Thus far, attempts to derivatize the carbonyl group with 2,4-dinitrophenylhydrazone (DNPH) have been unsuccessful. Recently, this reaction was tried again using DNPH in acetonitrile, but this also failed to provide the derivative. When a solution of PsON hydrochloride was made basic and passed through a sulfonic acid solid phase extraction column, GC/NPD afforded four peaks, one of which corresponded to that observed when PsON was made basic, extracted and injected into the GC. This indicates that one approach may consist of direct analysis of PsON by GC without derivatization, although there appear to be many possibilities for further reactions if this approach is adopted.
- C. **Plans:** Meet with selected individuals to formulate additional approaches to the analysis of PsON.
- D. **References:**

Keene, C. K. Notebook No. 8946, pp. 51-52.

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